

XI.—On the Amount of Substance-waste undergone by Insects in the Pupal State ; with Remarks on *Papilio Ajax*.
By RAPHAEL MELDOLA, F.C.S.

THE observations herein recorded are preliminary to an experimental inquiry into the law which governs the size of the individual in species undergoing complete metamorphosis. Being unable to continue the investigation until next season, when some species going through its larval and pupal stages within the season will be procurable, I deem it advisable to make known the results of my experiments so far as these are at present conducted.

The ideas that have led to this inquiry are briefly these:—From the period of its emergence from the egg to the assumption of the pupal state the larva of an insect undergoing complete metamorphosis continues to feed and to increase in bulk ; in this stage the insect exerts but little activity, so that nutrition is in excess of waste and a considerable surplus is left for growth. With the pupal stage comes a period of quiescence, when the substance-loss due to activity is reduced to a minimum.

The experiments of Newport* have shown that in the pupal state respiration still goes on, though to a diminished extent, so that the carbonic acid and water excreted by the pupa, being uncompensated by food, must be a dead loss of matter to the insect. Several weighings made by Newport prove the truth of this statement. Thus, there being gain of matter in the larval state and loss during the pupal stage, and there being, moreover, undoubted variation in size among the individuals of a species (apart from sexual difference in size), it occurred to me as probable that one of the laws governing individual size was to be derived from the facts above set forth ; for from these we might fairly expect that the size of an individual (all disturbing factors being eliminated) would be, *cæteris paribus*, inversely proportional to the ratio of the pupal to the larval period, or directly proportional to the ratio of the larval to the pupal period.

Such being the law arrived at *à priori*, I determined to submit it to experimental investigation when a favourable opportunity presented itself. In the mean time, when searching for recorded facts bearing on the subject, I became acquainted with the interesting experiments of Mr. W. H. Edwards † upon

* Phil. Trans. 1836 & 1837, vols. cxxvi. & cxxvii.

† 'Butterflies of North America,' part ix., Dec. 1871. I am indebted to my friend Mr. A. G. Butler, F.L.S., for the loan of this work and also for specimens of *P. Ajax*.

the breeding of *Papilio Ajax*; and these seemed to furnish data fitted for testing my conclusions.

Papilio Ajax is a polymorphic species inhabiting North America, presenting the three following well-defined forms:—

1. *Papilio Ajax*, var. *Walshii*, Edwards.
2. ———, var. *Telamonides*, Felder.
3. ———, var. *Marcellus*, Boisduval.

These three forms differ in many specific characters, and were long recognized as distinct species. They form a series graduating in size, *Marcellus* being the largest and *Walshii* the smallest. The first to appear on the wing in the year is *Walshii*; this is followed by *Telamonides*, and this in its turn by *Marcellus*. The larvæ of *Walshii* are described by Mr. Edwards as being very uniform in colour and marking; those of *Telamonides* are similar to *Walshii* "up to the second moult, after which there is a wide divergence, some retaining a resemblance to *Walshii*," others presenting several variations. "The larvæ of *Marcellus* combine the variations of *Walshii* and *Telamonides*." The specific identity of the three varieties was proved by rearing them all from one batch of eggs. Mr. Edwards thus sums up the results of his whole series of observations:—" *Walshii* produces *Walshii*, *Telamonides*, and *Marcellus* the same season, and its own type in the spring; *Marcellus* produces successive broods of *Marcellus* the same season, and occasionally *Telamonides* (individual taken in September 1870), and the last brood produces *Walshii* and *Telamonides* in the spring; and whenever any of the chrysalids of either brood of *Marcellus* pass the winter they produce the other two varieties, and probably sometimes their own type (individual taken in April 1867). The chrysalids of *Walshii* that pass the winter of 1871-72 will produce *Walshii* or *Telamonides*." The most important difference between the varieties, so far as our present purpose is concerned, is that in the duration of the larval and pupal periods. The duration of the different stages in the three forms is shown below:—

	Egg.	Larva.	Chrysalis.	Total.
<i>Walshii</i>	7-8 days.	22-29 days.	14 days.	43-51 days.
<i>Telamonides</i>	4-5 " "	15-18 " "	11-14 " "	30-36 " "
<i>Marcellus</i>	4-5 " "	12-19 " "	11-14 " "	27-38 " "

Here, therefore, is a species presenting varieties differing in size and in the duration of their larval and pupal periods. It is easy to calculate whether any relationship exists between the size of the insect and the ratio of the pupal to the larval period; and on making the calculation I found that there was a relationship, but exactly the reverse of that which would be

anticipated from the conclusions previously set forth. This result is, I think, sufficiently curious to warrant insertion :—

Name of variety.	Ratio of mean pupal to mean larval period.	Ratio of mean larval to mean pupal period.	Mean expanse, δ .
<i>Walshii</i>	$\frac{14}{25.5} = 0.549$	$\frac{25.5}{14} = 1.821$	inches. 2.70
<i>Telamonides</i> . .	$\frac{12.5}{16.5} = 0.757$	$\frac{16.5}{12.5} = 1.320$	3.00
<i>Marcellus</i>	$\frac{12.5}{15.5} = 0.806$	$\frac{15.5}{12.5} = 1.240$	3.35

It is here seen that the size of the variety is directly instead of inversely proportional to the ratio of the pupal to the larval period and *vice versa*. Whether these results are due to mere coincidence or whether they are related as cause to effect I am quite unable to say. The calculations, however, point to the conclusion either that the pupal waste has no effect upon the size of the imago, or else that it is overbalanced by the action of other causes. The difference of size between *Walshii*, *Telamonides*, and *Marcellus* is therefore most probably correlated with those other differences (such as marking of imago, pattern and colour of larva, duration of egg-stage, &c.) which Mr. Edwards has shown to exist between these three varieties.

Double-brooded species appeared also to present at first sight data fitted for testing the conclusions arrived at; but further reflection convinced me that such facts as are known are so encumbered by factors of unknown value as to render their comparison useless. Thus the species of *Selenia* and *Ephyra* inhabiting this country are double-brooded, the individuals of the spring brood being in both cases larger than those of the autumnal brood. Now the spring brood passes the whole winter in the pupal state, while the autumnal brood passes only a few weeks in the summer in this condition. In this case *temperature* is the unknown disturbing factor. We know not the amount of the total loss either during the summer or winter pupal period; neither do we know the amount of the total gain of the larva during its vernal period of feeding or during its autumnal period of feeding. With *Selenia* also there is some difference of colour and marking between the two broods; so that we have here "seasonal dimorphism," with which, as in *P. Ajax*, the difference of size may be correlated. The European butterfly *Araschnia prorsa* is also double-brooded; but in this case the spring variety is the

smaller. In many species individuals sometimes fail to emerge at their proper period, and then remain in the pupal state until the following season. This commonly happens with *Eriogaster lanestris*, and occasionally with *Bombyx quercus*, var. *callunæ*; I have known it to occur also with *Sphinx ligustri*; and my friend Mr. W. Cole informs me that he has a pupa of *Papilio Machaon* now living (September 12th) which failed to emerge at the proper period. In all cases of this kind we are in complete darkness as to the respiratory activity of such dormant pupæ.

The experiments which I have now to record are extensions of Newport's experiments* upon the loss of weight in pupæ, and were undertaken with a view to ascertain the exact amount of the loss undergone by insects in this condition, and to gain also an insight into the extent of the individual variation in respiratory activity among pupæ of the same species exposed to the same thermal conditions.

The first weighings made were of a specimen of *Bombyx quercus* found in the larval state on Leith Hill on the 2nd of last June. It was nearly full-grown when found, and commenced to spin up a day or two after capture. About a week was allowed to pass before making the first weighing, in order to make sure that the larva had changed, and in order to diminish the risk of error due to the drying-up of the cast-off skin and of the cocoon. The weighings (made throughout in grammes) were repeated at intervals of seven days until the emergence of the imago, which took place on the 14th of July. This pupa was weighed in its cocoon, and the weight of the empty cocoon afterwards subtracted from each of the weighings. The following are the results:—

Table showing loss of weight in pupa of *Bombyx quercus* during periods of seven days.

Date of weighing.	Actual weight.	Loss.	Percentage of loss.
June 12th	1·381	—	—
„ 19th	1·280	0·101	7·31
„ 26th	1·257	0·023	1·79
July 3rd.	1·229	0·028	2·22
„ 10th	1·164	0·065	5·28

* This great physiologist proved that pupæ respired, by detecting the excreted carbonic acid; and he estimated the respiratory activity by determining the quantity of this gas. The estimation of the total loss of weight is essential to the present inquiry, because water, whether produced by the oxidation of hydrogen-containing tissues, or whether introduced into the system with the juices of the food-plant, is essentially a product of respiration, and counts, like carbonic acid, for substance-loss.

In the second column of the foregoing table the "actual weight" is the weight of the pupa alone. In the fourth column is calculated the percentage of loss upon the weight of the pupa at the commencement of each of the periods. I estimate that during the whole of its pupal existence this insect lost from 25 to 26 per cent. of its weight.

The next experiments were made upon six specimens of *Liparis dispar* bred from eggs supplied to me in 1872 by my friend Mr. G. W. Bird. These pupæ were, as before, allowed to remain some time before making the first weighing, and were then weighed quite naked, the cocoon and larva-skin being removed, so as to do away with all risk of error due to drying. The weighings were in this case repeated at intervals of four days, with the following results:—

Table showing loss of weight in six pupæ of *Liparis dispar* during periods of four days.

No.	Initial weight.	Serial weighings.											
	July 10.	July 14.			July 18.			July 22.			July 26.		
	Actual weight.	Actual weight.	Loss.	Percent. age of loss.	Actual weight.	Loss.	Percent. age of loss.	Actual weight.	Loss.	Percent. age of loss.	Actual weight.	Loss.	Percent. age of loss.
I. ♀.....	1.031	0.957	0.074	7.18	0.900	0.057	5.95	—	—	—	—	—	—
II. ♀ ...	0.938	0.898	0.040	4.26	0.859	0.039	4.34	—	—	—	—	—	—
III. ♀ ...	0.998	0.947	0.051	5.11	0.899	0.048	5.07	—	—	—	—	—	—
IV. ♂ ...	0.607	0.577	0.030	4.94	0.548	0.029	5.02	—	—	—	—	—	—
V. ♂ ...	0.429	0.414	0.015	3.52	0.400	0.014	3.38	0.382	0.018	4.50	0.112†	0.270†	70.68†
VI. ♀ ...	1.044	1.012	0.032	3.06	0.873*	0.139*	13.73*	0.690*	0.185*	20.96*	0.500*	0.190*	27.55*

* signifies that the pupa is dead. † signifies that the weight is that of the imago. Where no figure is supplied the imago has emerged.

The expressions "actual weight" and "percentage of loss" have in this table the same meanings as before. Specimen VI. was killed by exposure to ether vapour at the termination of the first period (July 14th), in order to compare the loss of weight due to simple drying up with that due to respiration. For this reason the dead pupa was always weighed on the same days with the living ones; and, as will be seen on reference to the table, it invariably lost more, from the period of its death, than any of the living specimens. All the specimens were kept together in the same apartment, so that they were exposed to the same temperature. Nos. I., II., and III. yielded perfect insects on July 20th; no. IV. emerged on July 22nd, and no. V. on July 26th. This last specimen happening to

emerge on a morning concluding a period, was weighed in the perfect state, and had lost more than seventy per cent. of its weight on July 22nd. This enormous loss is attributable to the increased activity of the imago, to the drying-up of the moist pupa-case, and to the ejection and drying of that red fluid which most Lepidopterous insects emit on their emergence from the pupa. Similarly Newport found that a pupa of *Sphinx ligustri* which weighed 67·4 grains had diminished to 34 grains when weighed 33 days after (the day after the emergence of the imago), having lost 52·1 per cent. of its initial weight. Specimen V. had lost on July 26th nearly seventy-four per cent. of its initial weight; but even this is smaller than the actual loss, because the first weighing was made more than a week after the changing of the larva, during which time the insect had been losing weight.

The next experiments were made upon living specimens of *L. dispar* in the perfect state. Three females and one male were weighed in boxes on July 10th, and then weighed again four days after, with the following results:—

Table showing loss of weight in four specimens of *Liparis dispar* (perfect insects) during a period of four days.

No.	July 10th.	July 14th.		
	Actual weight.	Actual weight.	Loss.	Percentage of loss.
I. ♂	0·118	0·092	0·026	22·03
II. ♀ . .	0·451	0·414	0·037	8·20
III. ♀ . .	0·514	0·460	0·054	10·50
IV. ♀ . .	0·546	0·458	0·088	16·11

This last table shows in a beautiful manner the relation between activity and waste; for the male was very restless and fluttered about in its box, while the females were tolerably quiet. The loss in the male is to the mean loss in the females in the ratio of 22·03 : 11·60, which is very nearly that of 2 : 1. The mean loss in the females is to the mean loss in the four female pupæ in the same period of time in the ratio of 11·60 : 4·99.

Thus do these preliminary experiments prove that there is a loss of substance in the pupal state, that this loss is different in amount in individuals of the same species exposed to the same temperature, and that it is less in amount than that occurring in the same species in the perfect state or in a dead

pupa by desiccation—the comparisons extending in all cases over equal periods of time. They prove also, what is far more important to our present inquiry, that the variation in loss is sufficient in amount to lead us to expect the size, or at least the specific gravity, of the imago to be sensibly influenced by it.

Trusting that other observers may be induced to take up the inquiry next season (for in the multiplicity of observations there is strength), I propose to point out in concluding the various disturbing factors which would interfere with the experimental results. In any species, then, the size of an individual may be influenced by:—

1. *The natural difference in size between the two sexes of many species.* This factor is obviously eliminated by comparing only individuals of the same sex.

2. *The force of heredity—large parents tending to give rise to large individuals and vice versâ.* Eliminated by comparing only the offspring of a single pair.

3. *The varying supply of food to different individual larvæ.* Eliminated by supplying each larva with excess of its food-plant.

4. *The different amount of substance-waste in different individual larvæ due to dissimilar thermal conditions.* Eliminated by keeping the experimental larvæ at the same temperature. The amount of carbonic acid excreted by different individual larvæ of a species exposed to the same temperature is proved by Newport's experiments to vary but little. This factor might, in fact, by itself be neglected altogether, but becomes eliminated when eliminating the next factor.

5. *The different amount of substance-gain in different individual larvæ, due to individual variation in assimilative power.* Eliminated by periodically weighing the experimental larvæ, and comparing only the imagines from those which show a similar proportional increase during the same periods of time.

6. *The different amount of substance-waste in different individual pupæ, due to dissimilar thermal conditions.* Eliminated by keeping the experimental pupæ at the same temperature.

7. *The different amount of substance-waste in different pupæ (kept at the same temperature) due to individual variation in respiratory activity.* Newport's experiments and my own weighings prove that this is a variable factor. Its elimination can therefore only be effected by periodically weighing the experimental pupæ, and comparing only the imagines from those which in given periods of time undergo the same proportional amount of loss.

In these observations an ordinary chemical balance will be found most trustworthy for making the weighings.